MONOGRAPH 13

A STANDARD TECHNIQUE FOR TEMPORAL BONE PREPARATION

Makoto Igarashi

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A STANDARD TECHNIQUE FOR TEMPORAL BONE PREPARATION*

Makoto Igarashi

Monograph 13

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FOREWORD

The inner ear end organs are structurally fragile with the membraneous labyrinth weakly supported within the bony labyrinth. The technique of histological preparation must be directed toward preserving the anatomical relationship of the inner ear structures and minimizing post-mortem changes. The densely ossified petrous portion of the temporal bone must be decalcified and the inner ear spaces uniformly infiltrated with celloidin, all the while maintaining the histological integrity of the membranous labyrinth.

This monograph is intended as a guide in the preparation of good temporal bone slides which will make it possible to investigate the correlation between end organ functions and morphological findings. No fundamental histological information is included, however, as that appears in other publications.

The techniques described herein have been found to render the most consistently satisfactory temporal bone preparations. The procedure is one of the most standardized and is that routinely used in otological research laboratories at Henry Ford Hospital, Detroit; Massachusetts Eye and Ear Infirmary, Boston; and U. S. Naval Aerospace Medical Institute, Pensacola, Florida.

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ANIMAL PERFUSION

The intravital cardiac perfusion technique is the one necessary for elimination of post-mortem autolytic changes. The technique was illustrated early in this century by Eckert-Möbius (1926). The perfusion apparatus used in all laboratories is essentially the same (Figures 1-3).

This technique requires the use of the following supplies:

1. Perfusion apparatus: aspiration bottles, box, tubing, Y connector, clamps, cardiac cannula, et cetera
2. Animal perfusion board
3. Blood clearing solution; most commonly used is normal saline solution
4. Fixative solution: Heidenhain-Susa, Wittmaack, Formalin, et cetera
5. Dissecting set: surgical knife blades (Parker #10, #11, #15)
   blade holders
   hemostats (3)
   bone cutters (Liston, small)
   dissecting scissors
   iris scissors
   towel clip
   black silk suture
   bone rongeurs (small and large)
6. Specimen jar: 1 pint size (preserving jar).

About 500-700 ml of both normal saline and fixative solution are needed for medium to large cats and less for squirrel monkeys and smaller animals. The clearing solution and the fixative solution should be determined on the basis of the experimental purpose. Flush out the air from the tubing from the fixative bottle first, then open the clamp of the tubing from the saline bottle to flush out both air and fixative solution (Figures 2, 3).

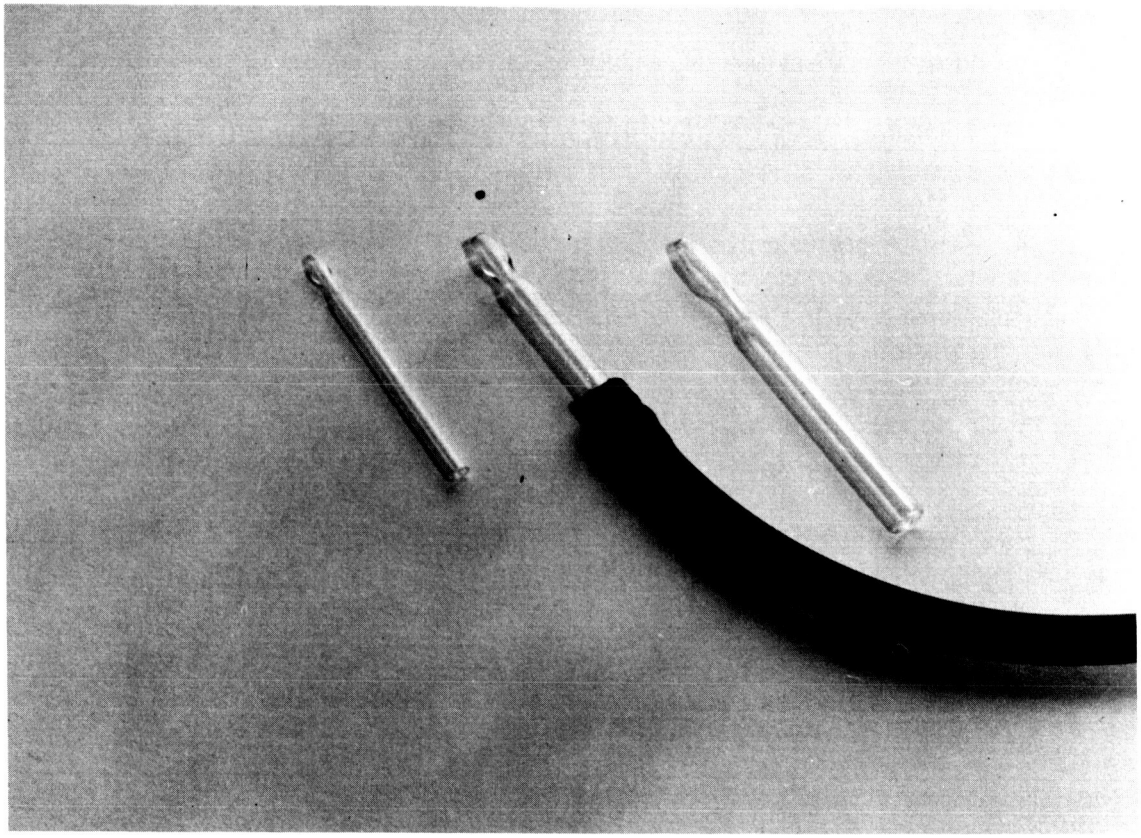

Figure 1

Cardiac cannulas (glass) for perfusion fixation in squirrel monkeys (left), cats (center), and larger animals (right) 2/3 x

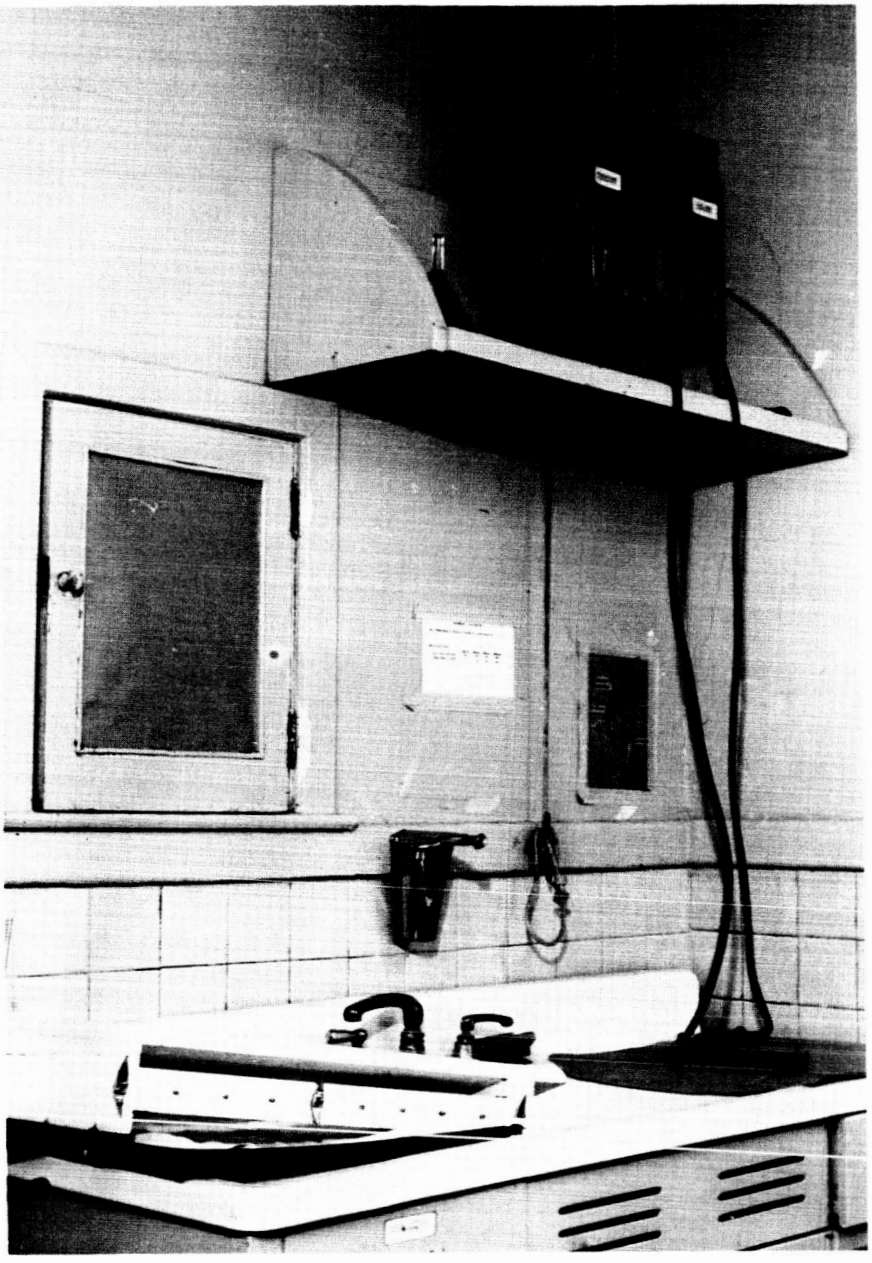

Figure 2

Animal perfusion apparatus used at Henry Ford Hospital, Detroit

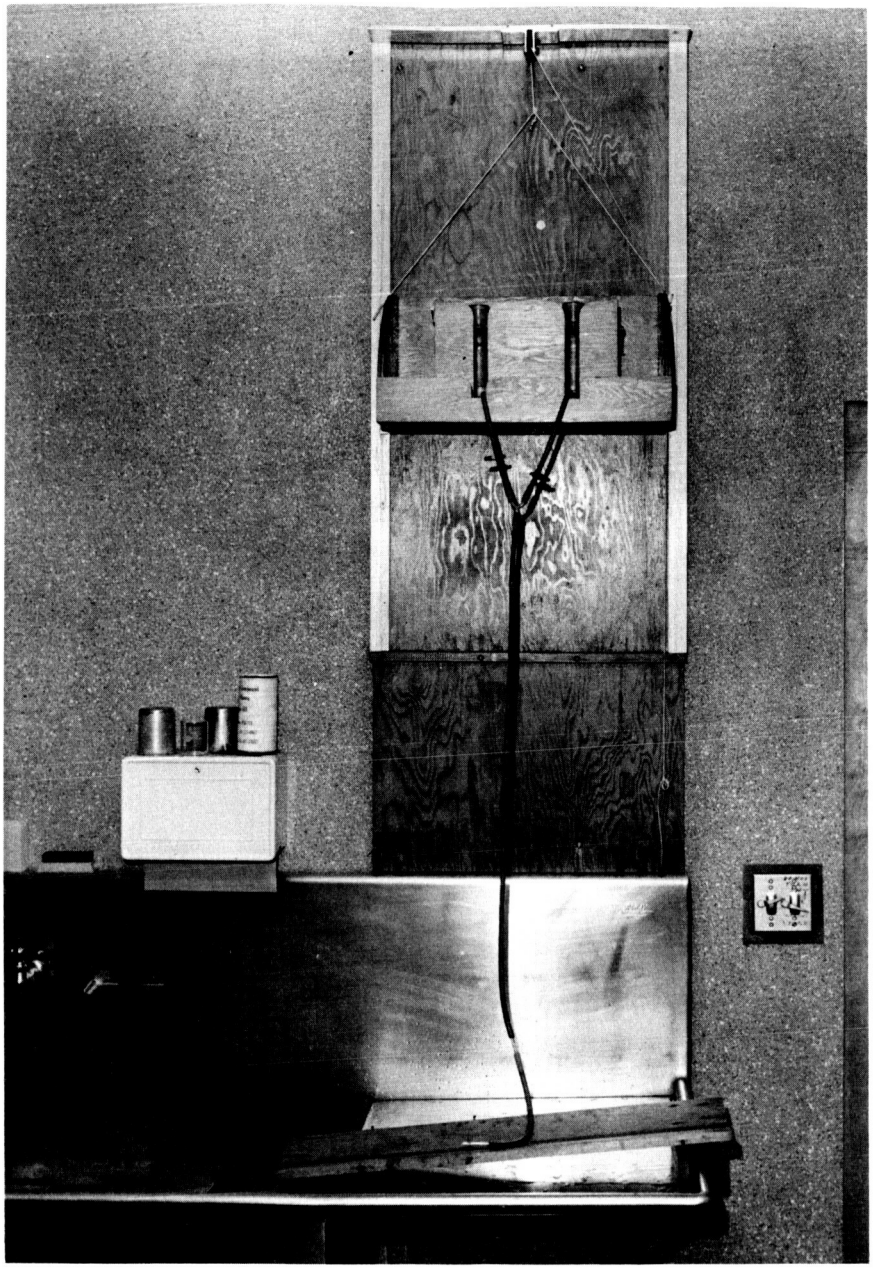

Figure 3

Animal perfusion apparatus. Installed on wall with rails. Good for rhesus monkeys to guinea pigs. (U.S. Naval Aerospace Medical Institute, Pensacola, Florida.)

Leave this clamp slightly open to permit a very slow flow of saline through the tubing while the animal is being prepared.

Check the dissecting set, jars, dissecting board, et cetera, again, and anesthetize animal deeply. The intraperitoneal injection of sodium pentobarbital (about 2 cc for the squirrel monkey and about 3–4 cc for the cat) is commonly used for this purpose.

The animal is secured in supine position on the board. The chest is opened wide by either a U-incision or a longitudinal incision, and a self-restraining retractor is needed to keep the thorax open. After opening the chest cavity, it is important to proceed with the perfusion as quickly as possible, otherwise a possible agonal change might occur. The pericardium should be removed, with iris scissors and small forceps, up to the ascending aorta area. Care should be taken to avoid cutting the auricle or large vessels. The point of the left ventricle is punctured with a knife. A gush of bleeding is expected at this time. The cardiac cannula is introduced through this opening into the ascending aorta. A previously placed black silk suture or specially prepared clip will secure the cannula in the aorta. It is very important to make certain that the point of the cannula is located in the ascending aorta and not deeply within. Clamp the descending aorta with a hemostat to facilitate the procedure. Open the clamp of the tubing from the saline bottle, and incise the right auricle with scissors.

Normal saline solution is not necessarily warmed before use. Good results could be obtained with cold saline. Small branches of spiral arteries and small vessels in the stria vascularis, et cetera, in the sections will appear to be free from blood cells when adequate fluid pressure has been applied during the perfusion. Since the fixative solutions usually irritate the eyes and the upper respiratory tract of the experimenter, good air ventilation is needed.

Watch the saline coming out from the right auricle. When the fluid becomes free of blood, close the clamp from the saline and immediately open the clamp from the fixative solution. If a very strong fixative such as Heidenhain-Susa solution is used, usually the jaw and neck become stiff immediately. Use all of the fixative solution in the bottle. After completing the perfusion, start to dissect for the temporal bones.

Make a V-incision along the submandibular line, excise all the soft tissue from the base of skull, and separate the head from the first cervical vertebra. Make a dorsal midline incision from the frontal area to the foramen magnum, then excise all soft tissues, including the skin, temporalis muscle, and auricles, cutting the external meatus with a knife. The calvarium is removed piece by piece with rongeurs, starting from the foramen magnum and extending anteriorly. Remove the tentorium and whole cerebrum without damaging the cerebellum. Gently retract the cerebellum anteriorly and cut the eighth nerve trunk at the orifice of the internal auditory meatus, or, leave a part of the cerebellum with the temporal bone. The squamous portion of the temporal bone is removed with rongeurs down to the petrous. After separating the temporomandibular joint with a bone cutter, a block of two temporal bones, which is connected by the base of skull, will become free. Remove as much as possible of the soft tissue. If the temporal bones should be processed separately, use a bone cutter to separate the two sides. If the animal has a bulla, its bony wall should be opened widely by rongeurs. The bony septum between the bulla and the middle ear cavity should also be removed. Since this septum is located so closely to the middle ear structures, a fine instrument is required to remove it.

The whole procedure should be done as quickly as possible. A person who is well trained usually can accomplish this within a few minutes after the decapitation. The specimen should be immersed immediately into cold fixative (about 300-400 ml). A capillary pipette or a syringe with a long, dull-pointed needle is used to flush out the air from all cavities of the specimen; thereafter the preserving jar should be tightly capped and kept in a refrigerator during the fixation period. The trimming of the excess of soft tissue should be done during the fixation period also.

SCHEDULING AND RECORDING

A main protocol follows each individual case schedule with a brief history included, and daily processes are recorded on a calendar. This double scheduling is strongly recommended. It is suggested that these case schedules be reviewed occasionally and that the stock amount of all items necessary to process the specimens be checked.

The specimens are usually so valuable and unique that it should not be essential to pay too much consideration to the economy of materials, labor, or time. The emphasis should always rest on preparation of the best slides.

The preferred way of preparing the slides is for each particular case to be handled by only one person. However, half of the responsibility may be delegated to only one particular individual for sectioning, staining, and completion of the specimen. In that case, another could handle the preliminary procedures of fixation, decalcification, neutralization, dehydration, and embedding.

FIXATION

Temporal bones removed from the perfused animal or secured from the human cadaver should be placed immediately into a preserving jar (one pint size) with a spring clamp. The same preserving jar will be used until the completion of celloidin hardening and will be reused for other cases. However, the rubber gaskets of the jar should always be checked and changed frequently. A generous amount of fixative solution should be in this jar; for animals use a minimum of 250 cc, and for humans a minimum of 350 cc.

It is recommended that these jars be placed in a refrigerator during the fixation period. To remove all air bubbles from the tissue, the jar should be gently shaken several times each day.

When using Heidenhain-Susa fixative, an average of two days for perfused animal bones and two to three days for human bones is required for fixation. With 10% formalin fixative, seven to ten days are required for animal bones and ten to fourteen days for human bones. The preferable procedure is to use 20% formalin for a period of one to two days prior to using 10% formalin.

Excessive tissue should be trimmed off before fixation is completed.

There are two different ways of preparing the Heidenhain-Susa fixative:

1. Solution I	Mercuric chloride ($HgCl_2$)	4.5 gm
	Sodium chloride (NaCl)	0.5 gm
	Distilled water	80 cc
Solution II	Formaldehyde	20 cc
	Trichloroacetic acid	2.0 gm
	Acetic acide, glacial	4 cc

Mix together four parts of Solution I and one part of Solution II at time of use.

2. Mix all the above-listed chemicals, except formaldehyde, with the water to make Heidenhain-Susa stock solution. Mix four parts of this stock solution and one part of formaldehyde at time of use.

DECALCIFICATION

For good structural preservation of the inner ear end organs, a mild and gradual decalcification process should always be applied to these temporal bones.

For this purpose, 5% trichloroacetic acid solution is one of the most recommended acid decalcifiers. Five per cent formic acid or 1% - 5% nitric acid can also be used. Formic acid-formiate mixture is recommended by Kristensen; however, it might take twice as long to decalcify with this solution as with 5% nitric acid.

DECAL solution (commercial name; Omega Chemical Co., N.Y.) is a rapid and strong decalcifier, and end organ damage may occur. However, the author has utilized a diluted DECAL solution for many squirrel monkey and cat bones, with successful results.

Another good decalcifier is 10% EDTA (ethylenediamine tetraacetate) solution at pH 7.2-7.4, which acts slowly and safely. There are several different commercial brands of EDTA. This decalcifier is also useful for histochemical experiments.

The suspension of the specimen in the upper part of the solution by paraffin-coated black silk suture is strongly recommended.

The 5% trichloroacetic acid solution should be changed once every day for the first several days, and every other day thereafter. An electric shaking apparatus at a very slow speed can be used for the first seven days or so, but the continuous use of this apparatus is not recommended after this period. If a shaking apparatus is not available, the jars should be gently shaken manually several times every day. Also, the tops of the jars should be released at least once every day to relieve the inside gas pressure, then recapped tightly. The excessive tissue should be trimmed again, using very sharp razor

blades with extreme caution. To avoid the destruction of the important structures, this technique should be done only by a person knowing advanced anatomy.

The time needed for decalcification in 5% trichloroacetic acid solution varies greatly. The average time for human temporal bones is four to seven weeks, for cat bones three to four weeks, and a little less time is needed for bones of smaller animals, such as squirrel monkey, guinea pig, et cetera.

The end point of decalcification should be objectively detected before processing the specimen to the next step. The x-ray technique introduced by Hagens is quite useful for this purpose. Actually, it is essential after decalcification with the chelating agents, and a minimum of four to five x-ray films is needed for each specimen.

The needle test, which is the technique used to examine the hardness of the bony tissue by introducing a fine sharp needle into it, is useful in some cases. However, destruction of the important structures should be carefully avoided. Sometimes it is critical to test the middle (inner) part of the thick block specimen as the most important structures are usually located exactly in the middle of the temporal bone specimen. A sharp, fine needle is required.

There are several different methods used to detect calcium in the acid solutions. The most routinely used method is:

> Mix, in a test tube, one part of 5% ammonium oxalate solution and one part of 5% ammonium hydroxide solution with two parts of well-mixed acid solution from the specimen jar. Use a test tube with clear

water as a control and leave test tubes for at least fifteen to twenty minutes. If solution is clear, no calcium is present. Continue testing every other day. After three or more consecutive clear results, the specimen can be processed. Four clear results (about one week or more) are required for human bones.

After decalcification, the specimens should be 1) washed in running tap water for twenty-four hours, 2) neutralized by 5% sodium sulfate solution for twenty-four hours or more, and 3) processed through several changes of distilled water for twenty-four hours before dehydration.

DEHYDRATION

Because celloidin is ether-ethanol (1:1) soluble, water should be removed from the specimen before proceeding to the celloidin embedding.

For human and most animal temporal bones, the following procedure for dehydration is recommended:

30% ethyl alcohol	1 day
50% ethyl alcohol	1 day
70% ethyl alcohol	1 day
80% ethyl alcohol	1 day or more
95% ethyl alcohol	2 days (daily change)
100% ethyl alcohol	2 days (daily change)
Ether-100% ethyl alcohol	2 days (daily change)

Alcohol lower than 70% has little dehydrating activity; therefore, the time period required in 30% and 50% alcohol can be shortened to one-half day for small animal bones. (Also, the periods of 95% and 100% can be shortened to one day for these very small bones.)

Shaking gently several times a day is necessary during this procedure.

If the specimen is fixed in the Heidenhain-Susa solution, iodine solution (saturated solution of 95% alcohol and iodine crystals) should be added to the 70% and 80% alcohol until the color of the alcohol becomes champagne to scotch amber in appearance. This procedure is to remove the mercury crystals from the tissue.

The specimens must always be moved to higher (or lower) percentages of alcohol in gradual steps; otherwise, the inner ear structures will be damaged.

EMBEDDING

Parlodion-Mallinckrodt is a well-known celloidin preparate. To prepare celloidin solutions, weigh the parlodion strips (avoid any contact with water or grease) and add the necessary volume of 100% ethyl alcohol. Add the same volume of ether on the following day. (Example: 6% celloidin: 36 gms Parlodion + 300 ml 100% ethanol + 300 ml ether.) Large (1 quart) preserving jars are most suitable for celloidin solution stock. The jars should be cleaned and thoroughly dried before use. Celloidin solutions must be prepared at least one week for 3% and two weeks for 6% and 12% before the time of use. During this preparation period, use of an electric shaking apparatus at slow speed is suggested. It is important to make certain that the celloidin solution is homogeneously mixed before use. Do not discard a celloidin solution into a water sink as it will curdle.

For celloidin embedding, either 3% - 6% - 12% solutions, or 2% - 5% - 10% solutions, can be used.

The period for embedding is variable, depending upon the specimen. The lengths most recommended are:

	Human	Cat	Squirrel monkey and guinea pig
3% celloidin	3 weeks	2 weeks	2 weeks
6% celloidin	3-4 weeks	3 weeks	2-3 weeks
12% celloidin	4 weeks	3-4 weeks	3 weeks

(Before embedding in 3% celloidin, 1.5% celloidin may be used for a period of a few days.)

All air bubbles in or around the specimen should be removed by moving the specimen very gently during the embedding period. Before it starts to harden, the specimen should be positioned at the center of the jar, with the middle cranial fossa surface downward (cat and squirrel monkey).

Start the celloidin hardening process by loosening or removing the cover of the preserving jar, depending mainly upon the room temperature. The surface of celloidin will usually become tacky within two weeks or more, and about one month later the lower part of the celloidin will become hard enough (like rubber) to be poured over with chloroform. Gently pour about 50 ml of chloroform over the surface of celloidin. Checking the hardness of the celloidin by sticking a straight needle in is required before pouring the chloroform. Quick hardening procedure is not advisable because it frequently results in air bubble formation and an uneven contraction of celloidin.

Because this is a process of ether-ethanol evaporation, the time range of the hardening period depends mainly upon the volume of the celloidin and the atmospheric condition. If the room temperature is fairly low, the cover of the jars can be removed for a few hours. On the other hand, if the room temperature is high, or if the cover is removed too much, the oversurface of celloidin becomes hardened within a short period, while the bottom remains very soft. This rapid hardening will result in an uneven contraction, possible specimen distortion, and will prohibit the evaporation from the lower part of the jar. In that case, a little ether-ethanol should be poured over the surface to soften it again. The slow hardening procedure is strongly recommended at the present time.

After chloroform is added, celloidin usually takes several days to become firm down to the lower part of the preserving jar. Use a needle to check the firmness, and if it is like hard rubber, cut the block out from the jar. To store specimens for a long time, 80% ethanol should be used. Extensive trimming of celloidin around the specimen should be avoided. Leave at least one-half inch on all margins to provide enough support during the sectioning.

Although there are several different techniques of hardening, such as hardening celloidin without using chloroform, pouring into the stender dishes, using the desicator with chloroform, using a negative pressure, et cetera, the basic idea of each technique does not differ.

The author is not convinced that any other embedding materials can be used to prepare continuous serial sections of the large temporal bone blocks.

SECTIONING

As the mounting apparatus of the microtome has limited adjustability, before mounting on a mounting block, roughly decide the angle of cutting. The study of the orientation by using dried skulls of different animals is essential in learning correct mounting plane and angle. Make the bottom of the celloidin block flat, dissolve the lower part of the block a little with ether-ethanol, place some 18% celloidin on a metal mounting block, and put the celloidin block and the metal block with celloidin together. Press firmly. Apply a small amount of 18% celloidin around the base of the celloidin block and dip into chloroform (Figure 4 A,B).

The sectioning is the most difficult and important part of the entire procedure. There are different planes in which to cut the temporal bone: (1) horizontal, (2) vertical and perpendicular to the long axis of the petrosa, (3) vertical and parallel to the long axis of the petrosa. The advantages of each plane are discussed in the literature.

For graphic reconstruction of the cochlea, it is absolutely necessary to (1) obtain a good mid-modiolar section, and (2) cut through the cochlea in the same plane at a constant thickness (20 microns).

To make a horizontal series, keep the superior edges of the external auditory meatus and internal auditory meatus roughly in the same plane, tilt the anterior corner of the petrous bone a little lower (cat, monkey, and human), and start to cut. After the basal turn of the cochlea is reached, the cutting angle cannot be changed. Therefore, it is necessary to obtain the best cutting angle before reaching the cochlea, and preferably before reaching the superior canal ampulla. The cochlea should be cut through at a

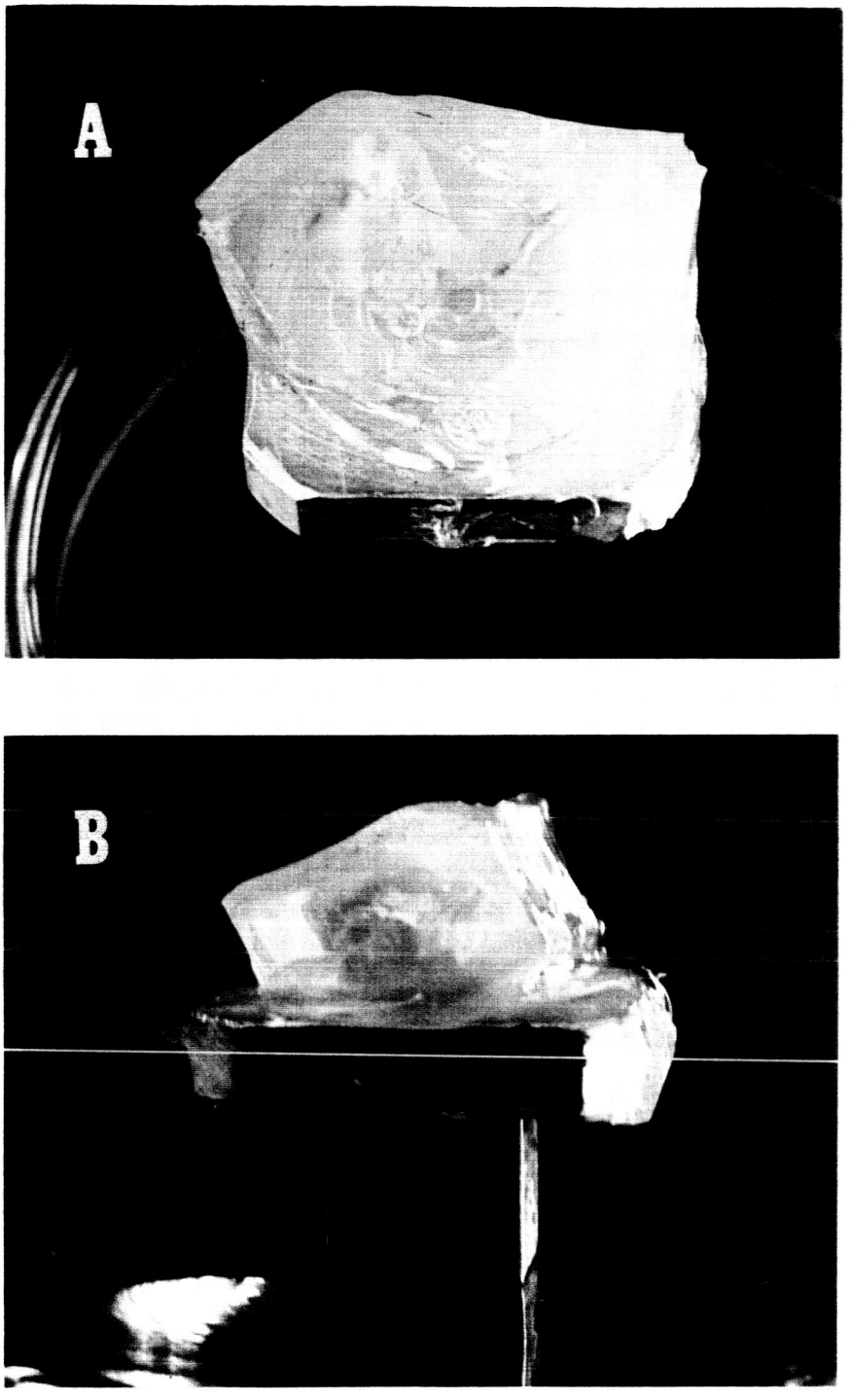

Figure 4

Upper (A) and side (B) views of celloidin block (cat) mounted on a metal holder.

Note the mounting angle. Bulla downward.

constant thickness. Some human or large animal bones are difficult to cut through at 20 microns. In that case, 25 microns might be used throughout, but the thickness cannot be changed until cutting through the cochlea is finished. It is true, however, that the thinner sections are more suitable for evaluation of histological conditions.

The precision sliding microtome (Spencer 860) with a 250 mm knife blade is common equipment for large temporal bone block sectioning. Since human bones are fairly large, it is necessary to use a raising metal block beneath the knife clamp. The most important instrument for sectioning is the microtome knife, and it should be handled with extreme caution. The knife edge should be microscopically checked and sharpened with a glass or metal knife sharpening apparatus. It is always important to keep the same honing angle while sharpening the blade. Use the handle and the knife's own honing guide for hand sharpening with a glass plate.

If a marking is applied to a two-ear specimen block before cutting to distinguish right and left, it is a tremendous help in mounting the specimens on the glass slides.

The sections should be cut under continuously dripping 80% ethanol, spread on the knife by a soft camel-hair brush, and placed on serially numbered onion skin paper for storage (Figure 5). Keep every 10th, or 5th (sometimes 20th) section in a separate dish for a pilot set (tracers) for inner ear reconstruction. The remaining stock sections (those other than a pilot set) should be placed in either a gauze or a paper package and kept in a preserving jar containing 80% ethyl alcohol.

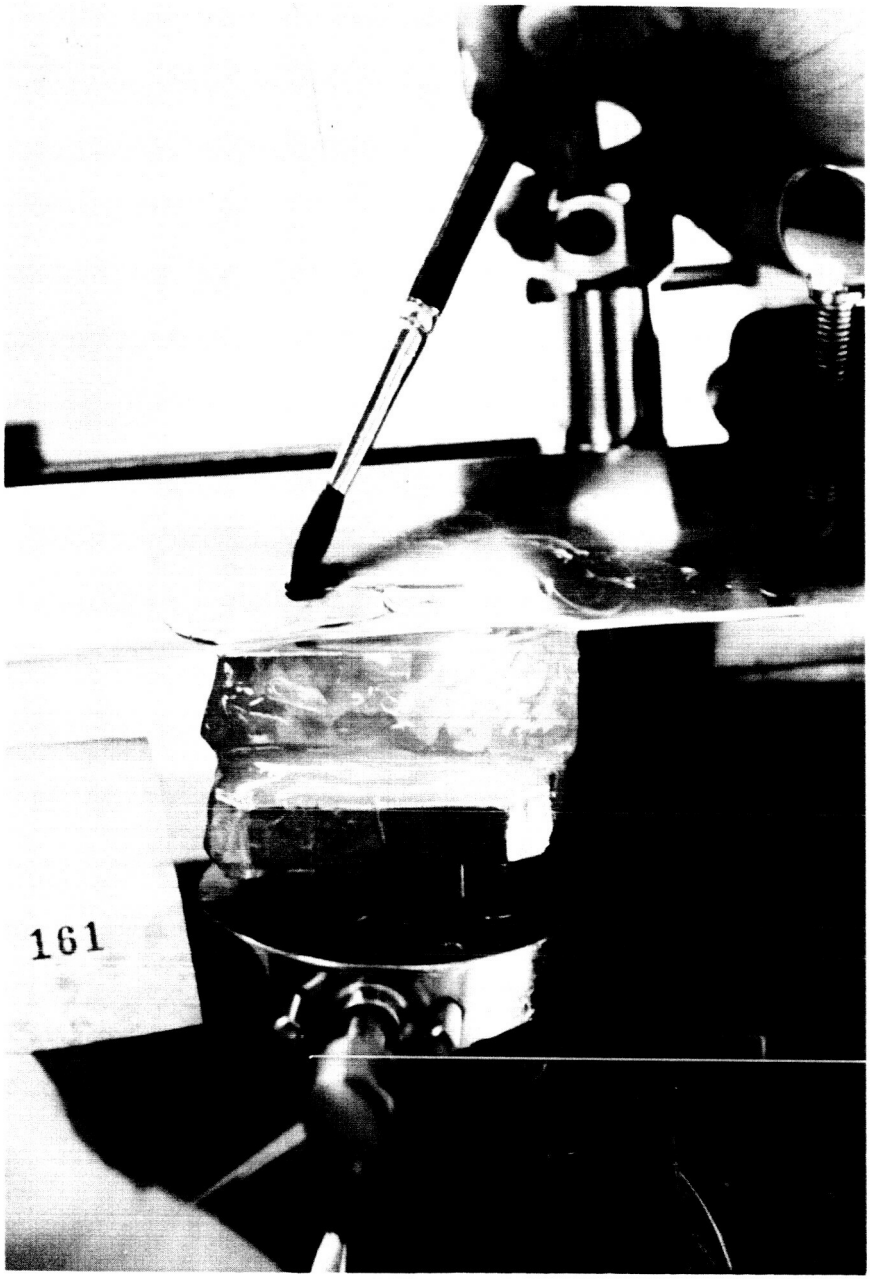

Figure 5

Sectioning technique with sliding microtome. Unroll celloidin before transferring onto the numbered onion skin paper.

All equipment should be cleaned and cared for after each use. The knife should be washed in tap water with a mild cleansing powder and soap, dried with 100% ethanol and dry gauze, and an oily film applied for storage.

Remarks regarding each particular sectioning procedure should be written into the protocol immediately after the action, for reference and possible future improvement in technique.

When any specimen is poorly infiltrated, the sectioning should be halted and the specimen should be re-embedded.

STAINING

Regarding the routine Hematoxylin-Eosin or Hematoxylin-Phloxin staining (bone and connective tissue staining), the following points are advised:

1. Stain in good contrast. This is important for observation and especially for microphotography.
2. Minimize the technical artifacts.
3. Keep the series in the correct order.
4. Stain the serial sections in constant color tone.

There are several typical staining techniques such as:

1. Mixed staining
2. Small group staining
3. Mounted staining

Mixed staining technique requires a series of large glass dishes (approximately 150 mm in diameter) and a greater volume of necessary staining solutions. Sections should be moved carefully from one dish to the next one by a bent glass rod or needle. Since the exact order of the series cannot be maintained in this technique, it is not recommended for one who cannot follow the correct order of the series. If several sections from the beginning and the end of the series (which are usually less important) are stained in a separate small dish, it will act as a staining pilot. This simple procedure may prevent a large variation in color while staining the more important sections.

For the small group staining technique, which is the one most recommended at present, several series of small dishes are needed. For example, if a series of six small dishes is available, put #1 section in the first dish, # 11 in the second dish, #21 in the third dish, #31 in the fourth dish, #41 in the fifth dish, #51 in the sixth dish, #61 again in the first dish, and so on. Therefore, sections #1, #61, #121, #181, #241, et cetera, will be processed together as the first group. Since these sections in the same group have definite anatomical differences, it is not difficult to distinguish them. It is, therefore, possible to maintain the correct order while staining (Figure 6).

In the first two techniques listed, specimens float in solution, and if the sections are too fragile, these techniques are not suitable. In the mounted staining technique, the specimens are mounted on glass slides, with egg-albumin, immediately after sectioning, and stained thereafter.

Figure 6

Small group staining technique (part of eosin counter-staining). Brown paper on table is for photographic purposes; background should always be plain white. Table vise is for the vise mounting technique.

ROUTINE HEMATOXYLIN-EOSIN STAINING

1. McCordick's solutions — 10-15 minutes

   McCordick's solution is 0.5 - 1.0 cc iodine solution in 100 cc of 95% alcohol.

2. 5% sodium thiosulfate solution (hypo) — 10 minutes

   Steps 1 and 2 are for Heindenhain-Susa fixed specimens only. Check for any remaining mercury crystals which appear as dark spots under the microscope after Step 2; if some remain, go back to Step 1. For formalin fixed specimens, start from Step 3.

3. Tap water — 5 minutes

4. Harris' hematoxylin — 40-60 minutes for humans; 20-40 minutes for animals

   It is important that hematoxylin be filtered immediately before time of use. The method for preparing Harris' hematoxylin is described in the literature. Overnight or long-period staining using very diluted hematoxylin (for example 1/100) is useful to obtain a more constant color tone. If very diluted hematoxylin is used, and the obtained color is adequate, decolorization could be omitted.

5. Tap water — 10-15 minutes

   Several changes

6. Acid alcohol

This is 0.5 - 1.0 cc of hydrochloric acid in 100 cc of 70% alcohol. Various time periods are required. The more acid added, the less time required before the excess hematoxylin in the celloidin disappears. Technical difficulty exists in determining when to move the specimens to the next step after an adequate period of time, and in keeping constant color in the entire series. It is necessary to have a plain white background.

7. Tap water — 5 minutes

Two changes

8. Ammonia water

This is 5-8 drops of ammonium hydroxide in 100 cc of distilled water. The tissue will become a bright blue with clear celloidin. A 0.5 - 1.0% lithium carbonate solution is fairly commonly used in place of ammonia water.

9. Tap water — 30-60 minutes

At least 3 changes

10. Eosin-Y (or Phloxine)

The timing for this counter-staining is very difficult to determine. It depends on the thickness of the specimens and the darkness of the hematoxylin staining. Stain a little darker to allow for later decolorization.

Add several drops of glacial acetic acid for each dish at the time of use to accelerate the staining. If the specimen has been too decolorized, it can be brought back to the stain.

11. 80% alcohol

    Three changes

12. 95% alcohol

    Two-three changes

    80% ethyl alcohol has a stronger decolorizing action than 95% does; therefore, change the 80% ethyl alcohol frequently when the color becomes red to avoid too much decolorization from the lack of clear visibility.

13. 100% ethyl alcohol with chloroform (1:1)

    In this step, float the entire specimen onto onion skin paper and trim the excess of celloidin with the paper by sharp scissors, and bring to the next step (Figure 7). This technique is far better for avoiding artifacts than is trimming celloidin on the slides.

14. Oil of Origanum — 40 minutes or more

    There are many variations of this clearing step, such as using equal parts of trepineal and xylene, et cetera.

    Color contrast is a matter of personal preference; however, good contrast is important for microphotographic purposes.

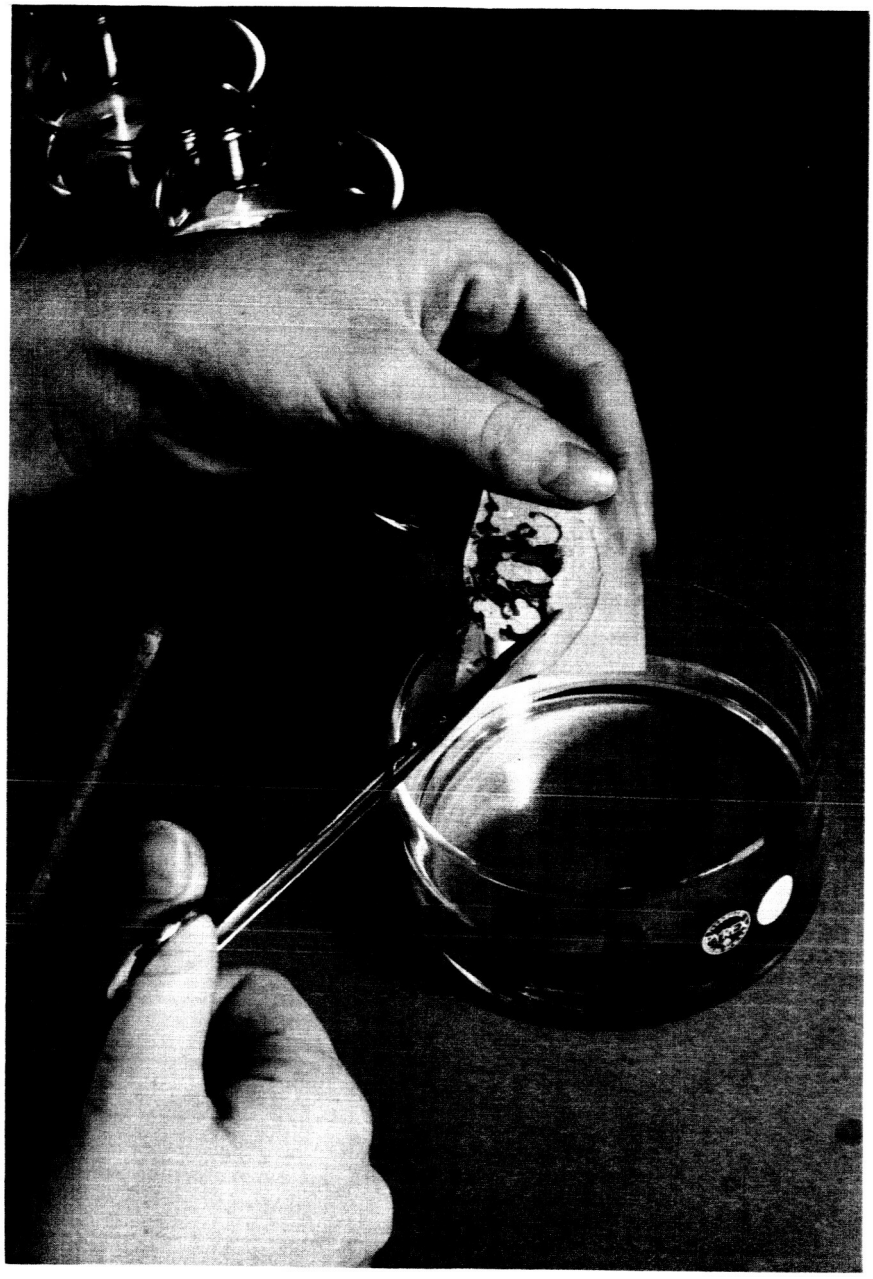

Figure 7

Final trimming technique after the staining. Specimen will be moved to Oil of Origanum after this procedure.

SLIDE-MOUNTING

To clean glass slides and cover slips, use acid-alcohol (1 ml of hydrochloric acid plus 100 ml of 70% ethyl alcohol), distilled water, 95% ethyl alcohol, then dry with a soft linen cloth or gauze.

To mount, use Balsam Canada with xylene (1:1 to 4:1). However, the choice of the mounting medium is a matter of personal preference.

It is very important to mount the serial sections in the correct position, at the correct angle, on the correct side, and in the correct order. Float the section in Oil of Origanum onto the glass slide, and take it out. Use a marked lined paper under the glass slide to indicate the correct position of the cochlea (Figures 8 and 9). Apply cover slips very gently. Use small lead weights (Figure 10) on cover slips for at least five to seven days. Thereafter, clean the slides with xylene, then water.

If a small table-vise is available, prepare several blotting paper blocks (each has about 10 sheets of blotting paper), put specimen on the glass slide and place between these blocks, press for three to five minutes in the vise, and cover with cover slips. A little harder Balsam than that used for ordinary mounting procedures is recommended for this technique, and there is usually no necessity to use lead weights, or to clean slides later. This technique is useful for a rush observation of the slide; however, it is important to make certain that celloidin sections are flat and adequate for microphotographic purpose.

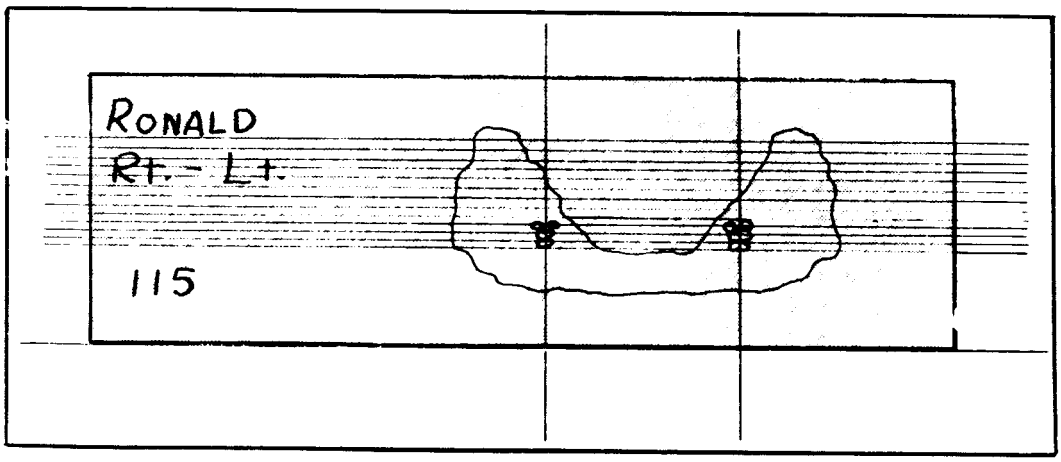

Figure 8

Use of a lined paper under a slide (cat).

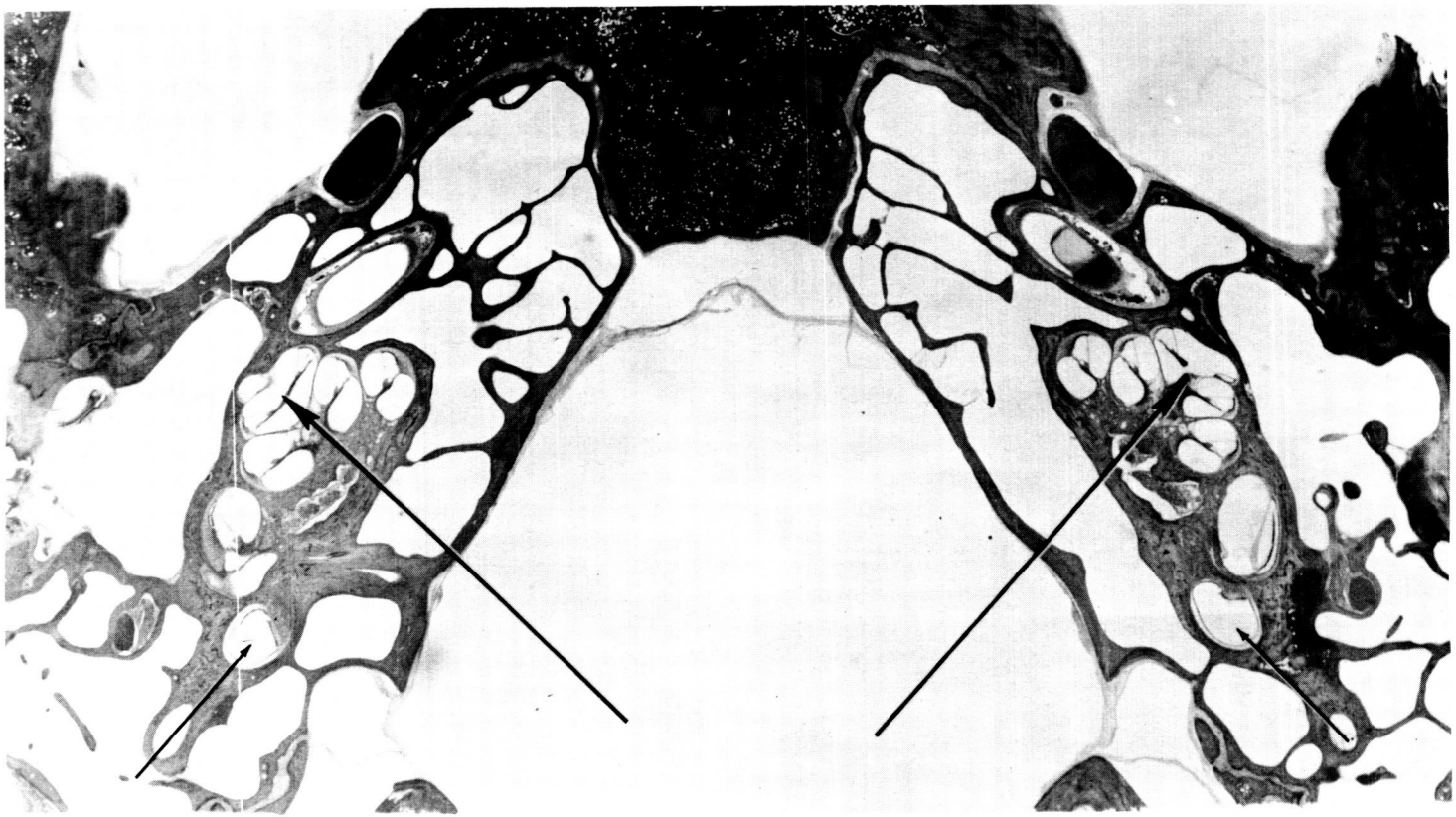

Figure 9

Mid-modiolar section of squirrel monkey. Open angles between modiolar lines (large arrows) and sagittal midlines are about 40° in the squirrel monkey although less than 10° in cat. Open angles of posterior semicircular canals (small arrows) are also different.

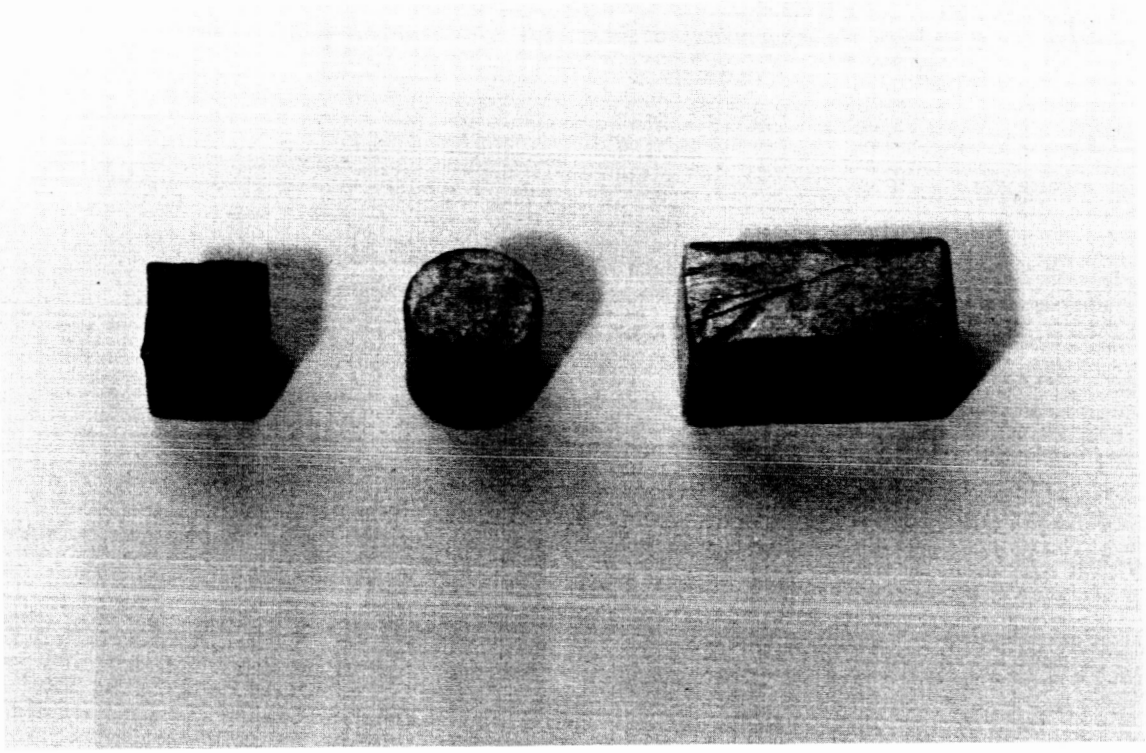

Figure 10

Lead weights of three different sizes. Square: 50 gm; round: 60 gm; rectangle: 180 gm. Weights should be arranged according to thickness of section.

It is difficult to follow the exact order of serial sections; therefore, putting numbers on glass slides should be done as a final step, especially after the group staining.

Sealing the cover slips by using a little amount of Balsam, clear nail polish, or any other suitable plastic-like sealer, is a useful technique to protect the slides from drying out.

MICROPHOTOGRAPHS

As a further guide to the reader, microphotographs, representative of temporal bone slides prepared by the procedures outlined in this monograph, are included in this section as Figures 11 to 16.

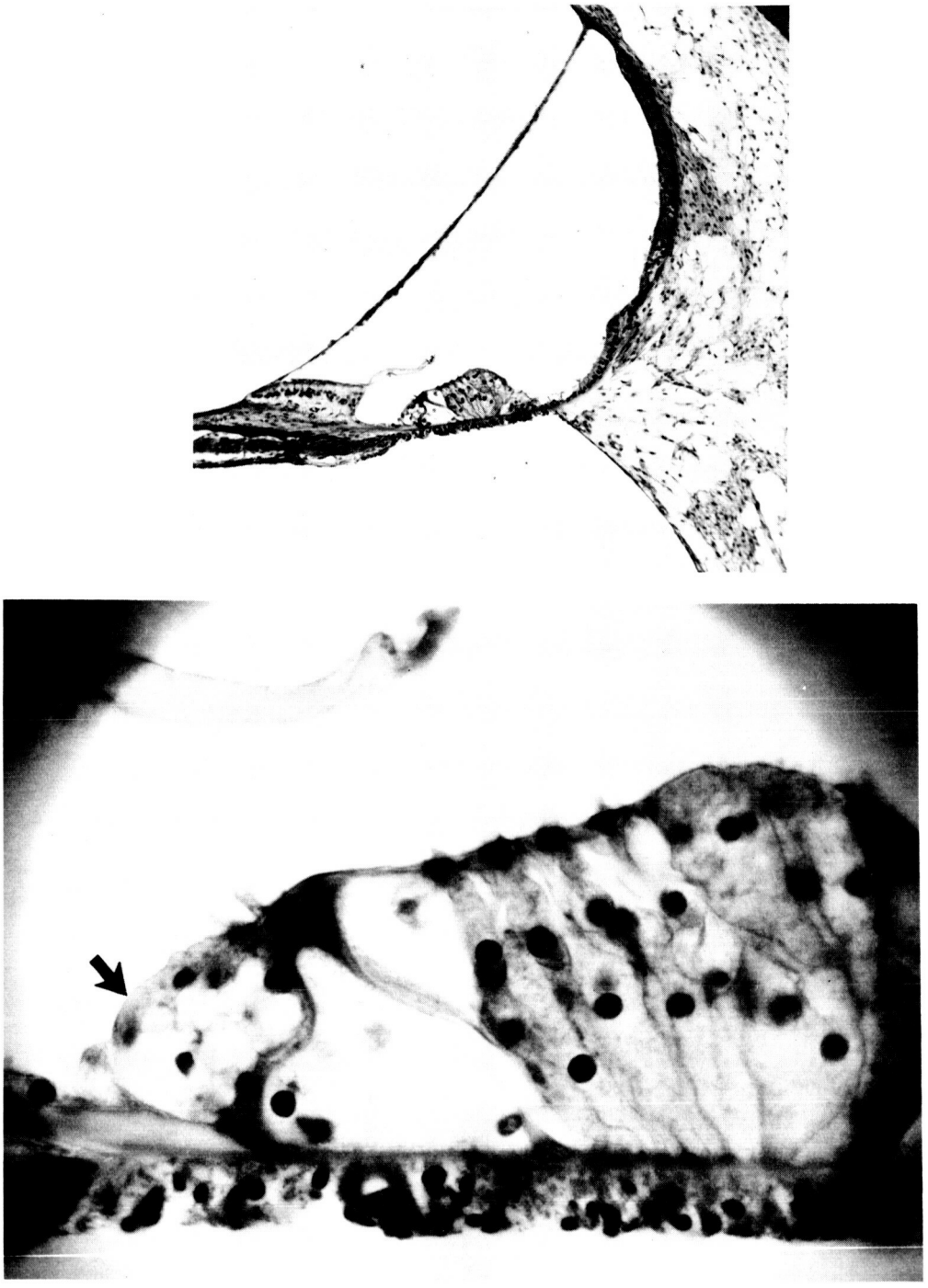

Figure 11

Low (top) and high (below) magnification views of normal human organ of Corti. Temporal bone was removed three hours post mortem, fixed (immersion) with Heidenhain-Susa fixative, and decalcified with 5% trichloroacetic acid solution. A post-mortem autolysis is seen especially around the inner supporting cells (arrow).

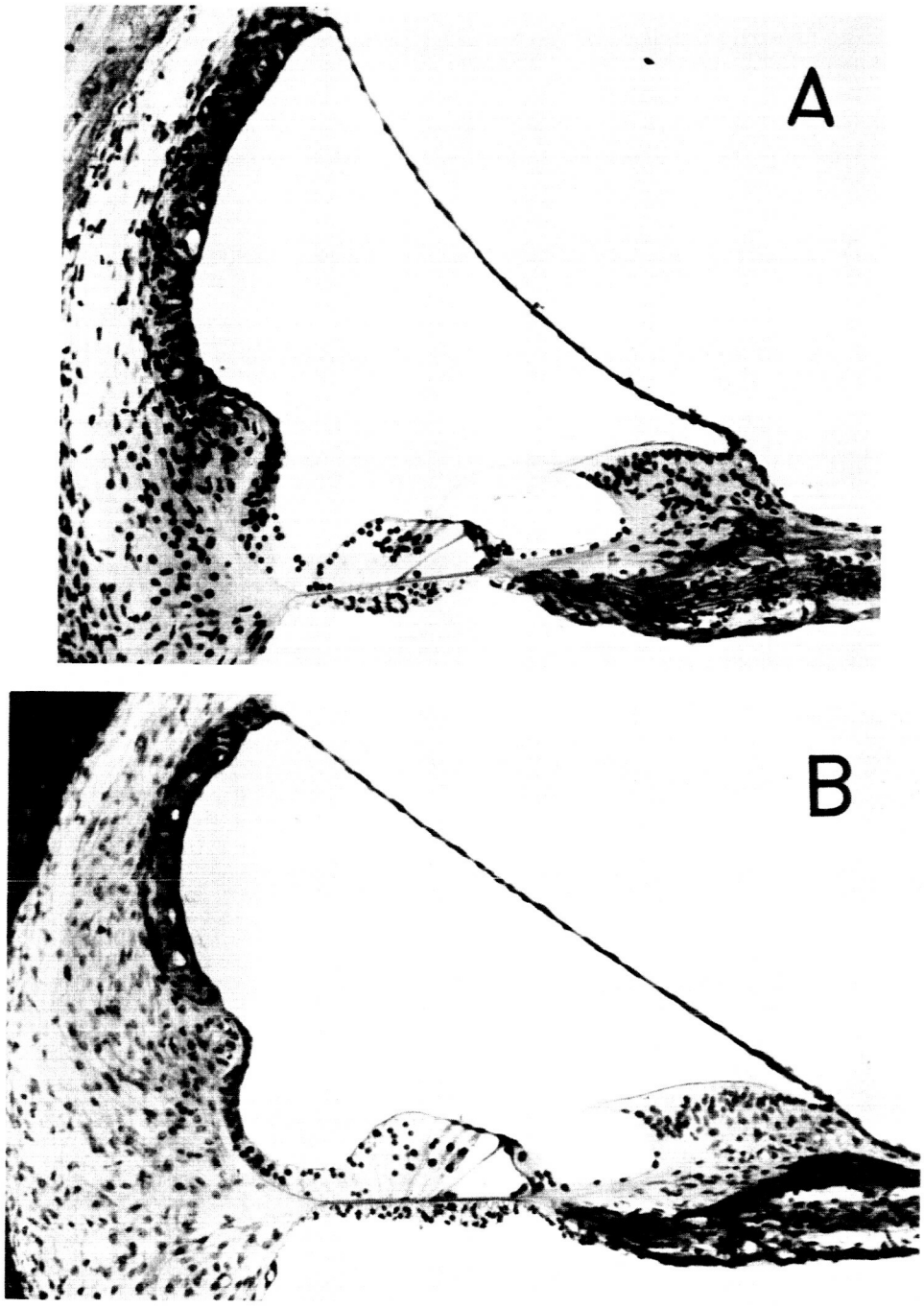

Figure 12

Normal organ of Corti from squirrel monkeys. (A) is fixed (intravital cardiac perfusion) with 10% formalin solution and decalcified with 10% EDTA. No acid was used for the preparation. (B) is fixed (intravital cardiac perfusion) with Heidenhain-Susa fixative and decalcified with 5% trichloroacetic acid. No post-mortem change is seen.

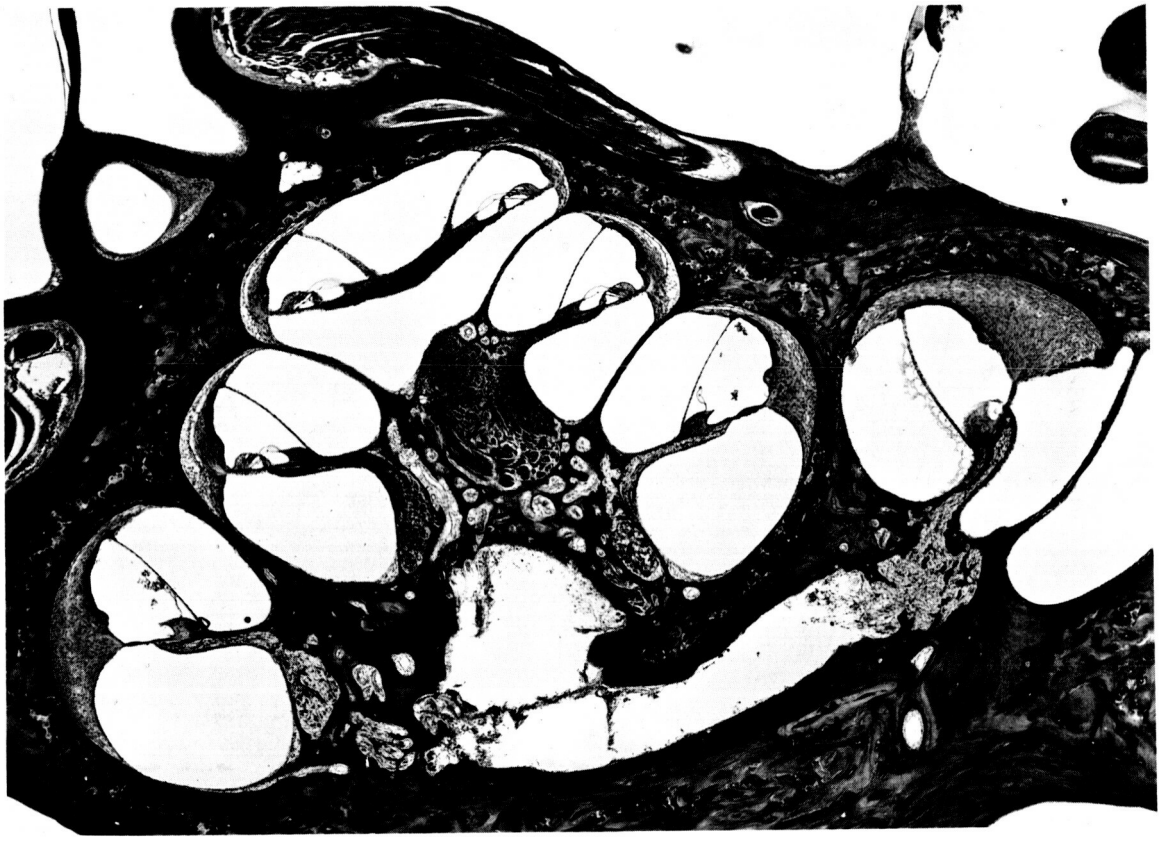

Figure 13

Entire view of the cochlea from the squirrel monkey after 4050 mg of systemic streptomycin sulfate injection. Notice the different status of organ of Corti and spiral ganglion in different turns. Pathology is severe in basal turns but not present in apical turns.

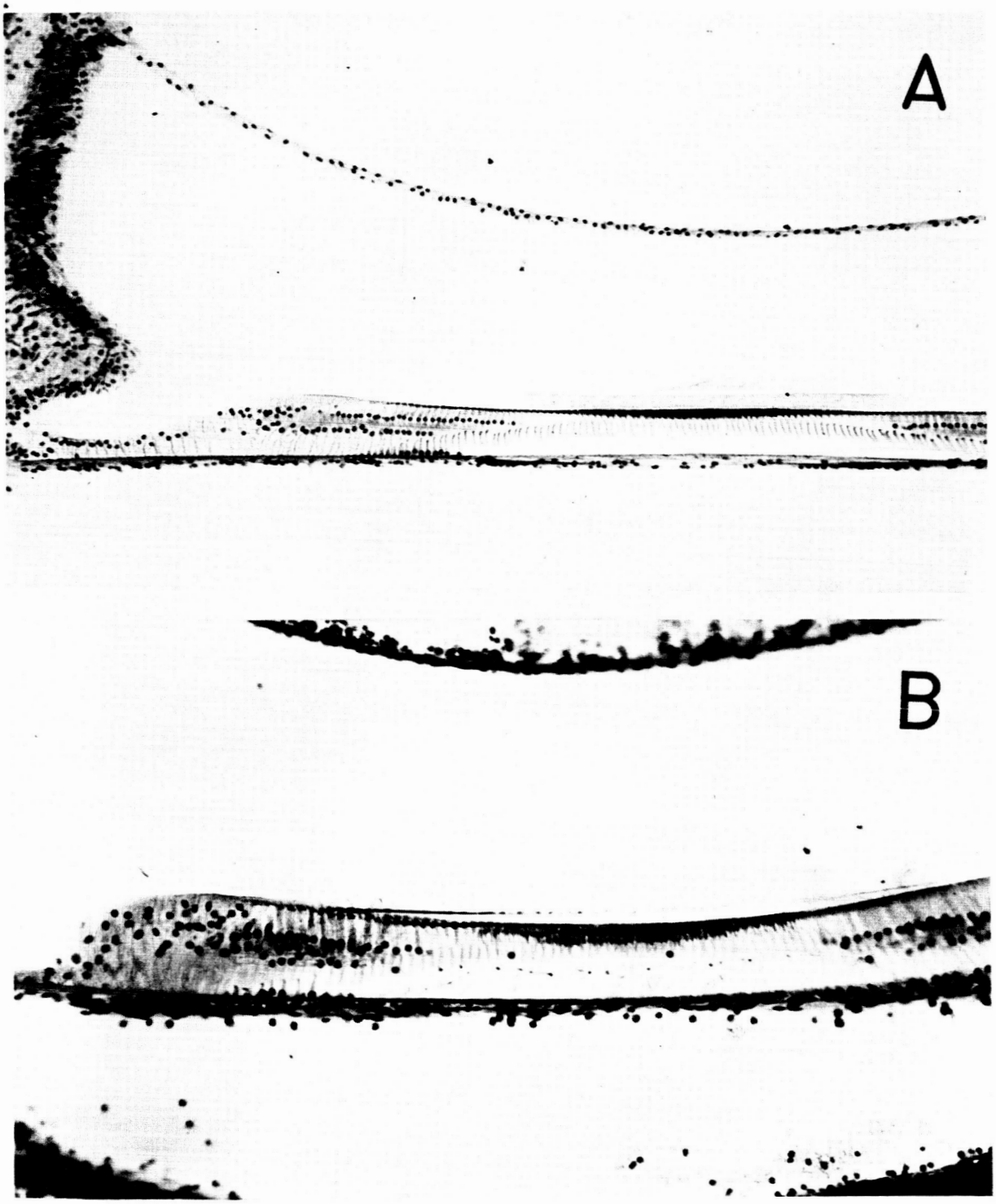

Figure 14

Two typical tangential sections of apical turns of the cochlea. (A): cat and (B): squirrel monkey.

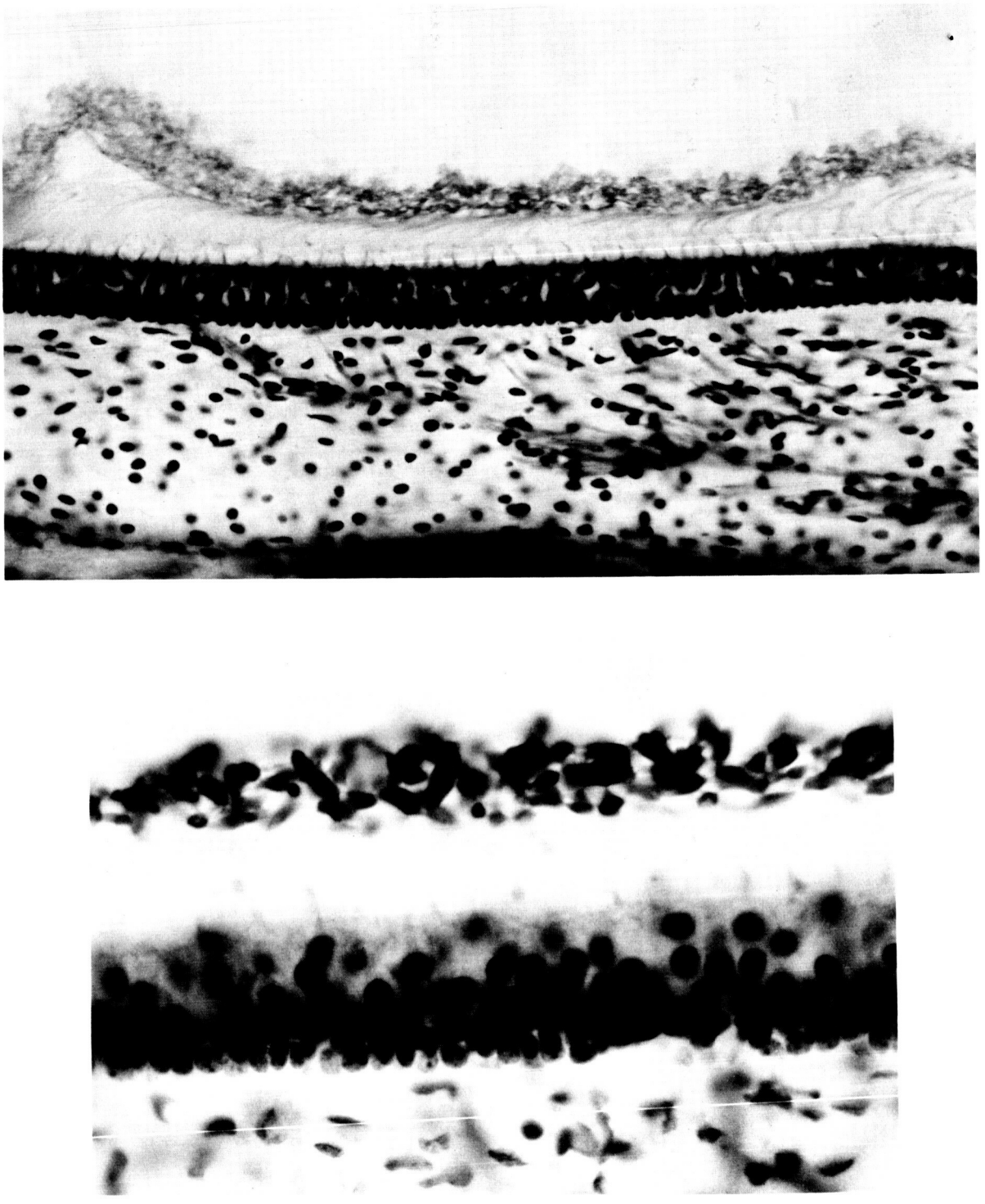

Figure 15

Low (top) and high (below) magnification views of normal saccular maculae from squirrel monkeys. Both specimens were fixed with 10% formalin and decalcified with 10% EDTA. Note the crystal shaped otoconia in high magnification view.

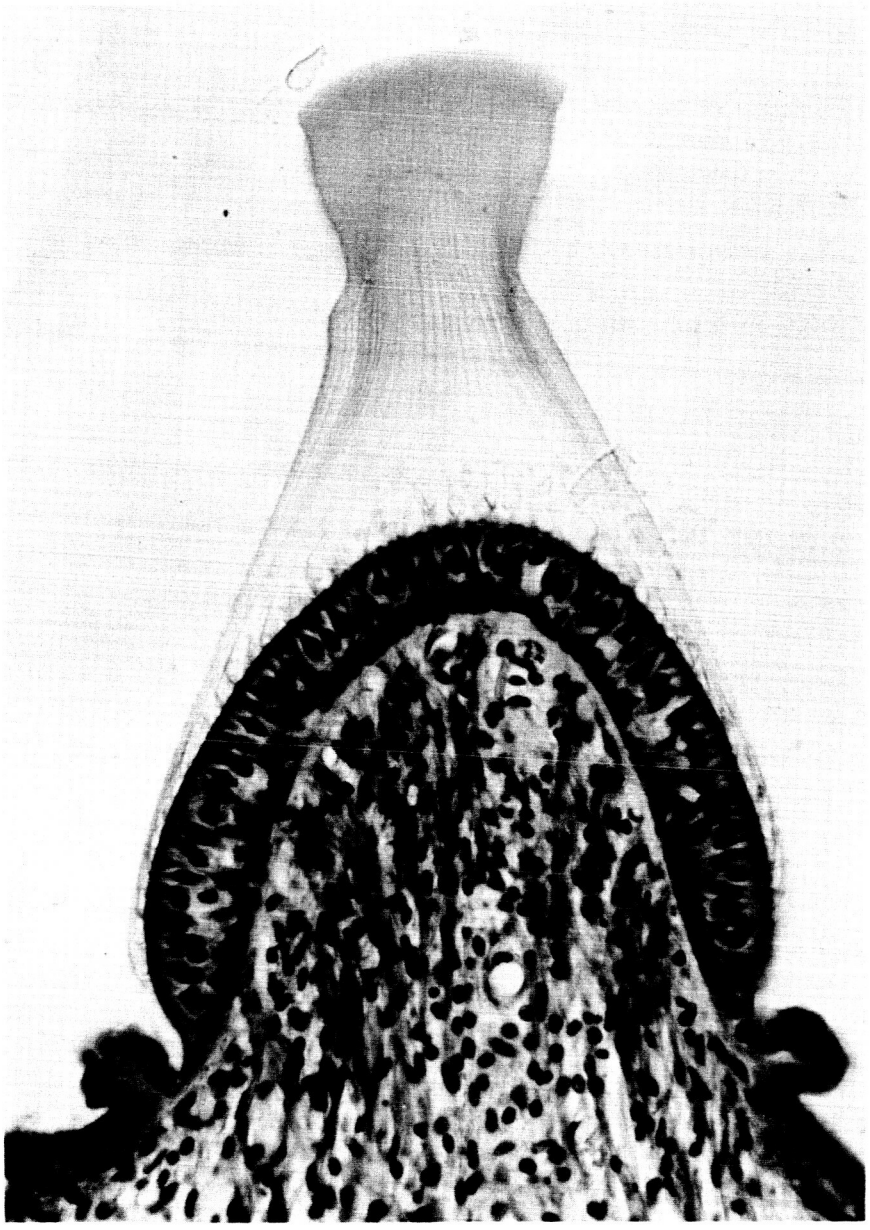

Figure 16

View of normal semicircular canal crista from a squirrel monkey. Specimen was fixed (intravital cardiac perfusion) with Heidenhain-Susa fixative and decalcified by 5% trichloroacetic acid solution.

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The inner ear end organs are structurally fragile with the membraneous labyrinth weakly supported within the bony labyrinth. The technique of histological preparation must be directed toward preserving the anatomical relationship of the inner ear structures and minimizing post-mortem changes. The densely ossified petrous portion of the temporal bone must be decalcified and the inner ear spaces uniformly infiltrated with celloidin, all the while maintaining the histological integrity of the membranous labyrinth.

This monograph is intended as a guide in the preparation of good temporal bone slides which will make it possible to investigate the correlation between end organ functions and morphological findings. No fundamental histological information is included, however, as that appears in other publications. The techniques described herein have been found to render the most consistently satisfactory temporal bone preparations.

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